

## The effects of repeated sessions of exercise on immune cells and cortisol in female athletes

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### Abstract

**Introduction:** Studies have shown that insufficient recovery between sessions causes immune suppression. The aim of this study was to investigate the effect of repeated bouts resistance and endurance exercise on blood leukocytes, lymphocytes, neutrophils, monocytes count and plasma cortisol levels in athlete girls.

**Materials and methods:** 9 athlete girls (ages, 20-25 years), participated in the study voluntarily. They performed two experimental protocols in two phases on two separate days. At first, subjects performed two bouts of resistance exercise (8 exercises with 65% 1RM) at 09:00 and 15:00. After one week rest, subjects performed two bouts of endurance exercise (60 min cycling on 65% HR Reserve) at the same time. Blood samples were taken before and after each bout and 1 hour after the second bout of exercise. Statistical analysis was performed using Kolmogorov-Smirnov, independent t-test and ANOVA with repeated measure.

**Results:** Repeated bouts endurance exercise significantly increased blood leukocytes, neutrophils, monocytes count and plasma cortisol levels, but repeated bouts resistance exercise only increased blood lymphocytes count significantly ( $P < 0.05$ ). Comparison of the two exercise types indicated that there was a significant difference in blood monocytes count ( $P < 0.05$ ).

**Conclusion:** The results showed no significant difference between repeated bouts of resistance and endurance exercise in a day on immunological variables and cortisol hormone.

**Keywords:** Immune system, leukocyte, cortisol

### Introduction

Body immunity system, like other physiological systems, produces a significant level of perturbations in response to a bout of exercise (1). A bout of heavy exercise could temporarily affect various aspects of innate and acquired immunity systems (2). In fact, strenuous physical activity produces perturbations in hemostasis of the body through significant changes in the number and distribution of circulating leukocytes, raising this number

to multiple times as this number in resting time, lasting even hours after the exercise (3).

A heavy bout of exercise consists of a major part of the athletes' drills during sport events, a period of immune system depression, which indicated an open window of immunity system lasting from 3 to 72 hours, thus increasing the probability that the athlete suffers infections (4). Although the real nature of

the response is not known, the close connection of the immune system and neuroendocrine system indicates that activation of endocrine neural system can potentially change the immune system, and major part of short-term response by immune system to a bout of exercise would be related to changes in the concentration of stress hormones mechanism (6). That is, it seems that a bout of athletic activity which brings fatigue could affect the immune system functions through triggering stress responses. A combination of fatigue due to insufficient recovery interval between bouts of exercises along with constant increase in concentration of stress hormones (especially cortisol) decreases the amount of circulating leukocytes. The decreased effect of white blood cells and other immune cells will bring decreased immunity activity against infections (7, 6). To reach peak performance, daily bouts of exercises by the professional athletes consisted of several bouts of acute exercise (8-10). Many researches indicated that different aspects of immune system experience changes after exercise temporarily (9, 12). Acute bouts of prolonged exercise without sufficient recovery intervals could result in chronic fatigue, reduced performance, and reduced immune system activity (9). Inherent and acquired immunity system could be suppressed temporarily after a bout of exercise or several bouts of prolonged exercises (12). The imbalance in competitive pressure and recovery has been suggested as one of immune system suppressions (13).

Recently, few studies have investigated the impact of several bouts of acute exercises on immunoendocrine responses (11-14). Results indicated that it is likely that several bouts of sport exercises induced more negative effects on immune system performance in short periods of time (3 to 18 hours) post exercise and that the second bout of exercise induced more pronounced

immunoendocrine disorders compared to the first bout of exercise (8).

The changes mechanism, at least in part, could possibly be due to metabolic stress in engaged muscles which consequently increases the activity of autonomic nervous system (ANS) and hypothalamic-pituitary-adrenal (HPA) axis, insufficient recovery and transitional effect of the previous bouts of exercise (9, 15).

Acute daily sportive activities would have a cumulative suppressive effect on immune system. Since the resting time between sport activities could be limited and when athletes do two bouts of exercise in a single day, the required awareness time for sufficient recovery after different sport activities before beginning the next bout is especially important.

With the athletes doing different sports (such as resistance and endurance) during events and exercises, awareness of possible differences in the effects of various sport activities on immunological and hormonal indices is quite necessary in timing of exercises. Awareness of physiological changes including changes in immune system indices could enable athletes to understand the necessary activities in recovery time to roll back changes to natural conditions. However, the effect of different types of sport activities and exercise plans on the immune system has been studied. No research has examined the effect of the type of daily sport activity on the immune system. With this in mind, the present study investigated the effect of two types of resistance and endurance activities with the same recovery time intervals between first and second bouts on the number of leucocytes, neutrophils, lymphocytes, monocytes and circulating cortisol in female athletes.

## Materials and methods

The sample population included female athletes aging 20-25 years old. After calling the female athletes for participation in the research across the city, 120 female

athletes participated in the study. Our criterion for being an athlete was the number of their bouts of exercises (3 bouts of resistance or endurance activity) during the three past years. All participants were quite healthy without any past record of hormonal disorders or any infectious or haematic diseases at least two months before the study. After briefing the participants on the research objectives and stages and filling in the statement of consent, participants entered the research process voluntarily.

First, the maximum power of the participants was estimated indirectly through the method cited in Brzycki et al., (17) and  $VO_{2max}$  was estimated through a 1.5 mile jogging exercise (18). Then, participants performed specific 2 bouts of exercise in two separate days. The first bout was a resistance exercise including 8 moves (bench press, shoulder press, Barbell biceps, lateral extension, and seated boat, back thigh with machine, front thigh, and leg press) in 3 sets of 8 repetitions, each set having 65 per cent of the maximum intensity at 9:00 AM and 15:00 PM. The resting time between sets was 3 minutes and it was 1 minute between moves. After a week, the participants performed the second bout of exercises including: first an endurance prolonged workloads of 60 minutes in bicycle ergometer with 65 per cent of age-predicted HRmax at 9:00AM and 15:00PM. To assess the intensity of endurance exercise, we calculated the activity as percent of maximal heart rate

(HRmax) in (Age minus 220) formula, and to monitor the intensity during activity, we used the Polar AXN300 Heart Rate Monitor/Outdoor computer with straps and alert system (Finland made). If the heart rate of a participant exceeded the limit, the computer signaled and the researcher adjusted the speed and heart rate. Bouts started with 5 minutes of warm-up, continued with the bout itself and ended with 5 minutes of getting cold. Blood samples were taken before and after each bout and an hour after the second bout. The blood samples were transferred to laboratory and the serum was separated from plasma. To measure the circulating cortisol, we used Monobind (US made) specific kit and Elisa method with the precision of 0.36 $\mu$ g/dl and cell counter machine (US made) to determine the number of leukocytes, lymphocytes, neutrophils, and monocytes. After determining data distribution normality, we used Kolmogorov–Smirnov test (K–S test) to examine intergroup variations in different test stages of ANOVA with repeated measure. We used Tukey post hoc test if there was a significant intergroup difference. To compare variations between two bouts of exercise, we used independent t-test with the significance level of  $p < 0.05$ . The data was fed into SPSS 16 for analysis.

## Results

Table 1 gives the mean and standard deviation of demographic features of the participants.

Table 1. Demographic features of participants.

Age (years)	Weight (Kg)	BMI Kg/m <sup>2</sup>	$VO_{2max}$ ml/Kg.min	Bench press (Kg)	Leg press (Kg)	Heart rate (Beat/min)
47.1 $\pm$ 2.2	73.4 $\pm$ 6.5	51.3 $\pm$ 4.2	14.7 $\pm$ 2.3	32.8 $\pm$ 4.3	86.1 $\pm$ 5.4	23.2 $\pm$ 6.6

BMI, body mass index.

Table 2 gives the number of leucocytes, its subgroups, and circulating cortisol following two bouts of endurance and

resistance exercises. Intergroup ANOVA with repeated measure indicated that repeated endurance exercises increased the

number of leucocytes after the second bout compared to its number before the first bout ( $P=0.026$ ). It also increased the number of leucocytes after second bout compared to its number before the same bout ( $P=0.022$ ). The number of neutrophils

was increased after the first bout compared to its number before the first bout ( $P=0.04$ ) before the second bout compared to its number before first bout ( $P=0.03$ ) and after the second bout compared to its number before the first bout ( $P=0.02$ ).

Table 2. The variations in the leukocytes, its subgroups, and circulating cortisol following two bouts of endurance and resistance exercises.

		9 AM	10 AM	15 PM	16 PM	17 PM
Leucocyte	Resistance	6914 ± 1924	7600 ± 1762	7414 ± 1624	7371 ± 1286	7500 ± 1714
	Endurance	5933 ± 1737	6966 ± 2124	*7083 ± 1938	8050 ± 2442	6066 ± 2828
Neutrophil	Resistance	4456 ± 1794	5078 ± 1711	4631 ± 1539	4645 ± 1234	4791 ± 1598
	Endurance	3512 ± 1112	* 4636 ± 2006	4641 ± 1904	4914 ± 1877	3677 ± 1701
Lymphocyte	Resistance	2234 ± 673	* 2278 ± 630	* 2631 ± 740	2527 ± 824	2453 ± 1117
	Endurance	2306 ± 730	2214 ± 216	2298 ± 394	2946 ± 887	2308 ± 1231
Monocyte	Resistance	127 ± 58	147 ± 85	114 ± 54	146 ± 44	152 ± 72
	Endurance	82 ± 62	74 ± 66	116 ± 58	* 167 ± 60	* 62 ± 44
Cortisol	Resistance	9.7 ± 5.6	6.9 ± 3.6	5.8 ± 4.8	5.7 ± 3.4	6.5 ± 3.9
	Endurance	9.9 ± 4.4	* 6.8 ± 1.6	* 3 ± 1	* 4.8 ± 1.7	* 6.9 ± 3.6

Data are shown as SD ± Mean; \* significance level ( $P<0.05$ ).

The number of monocytes was increased immediately following the second bout compared to its number before the second bout ( $P=0.029$ ) and 1 hour after the second bout compared to its number immediately after the second bout ( $P=0.025$ ).

The circulating cortisol concentration was lower before the second bout compared to its concentration before the first bout ( $P=0.005$ ); It was ( $P=0.046$ ) after the second bout compared to its concentration before the first bout; It was ( $P=0.002$ ) before the second bout compared to its concentration after second bout; It was ( $P=0.006$ ) before the second bout compared to its concentration after the first bout. The circulating cortisol was also higher 1 hour after the second bout compared to its concentration before the second bout ( $P=0.032$ ).

With the resistance exercises, only the number of lymphocytes was higher after the first bout ( $P=0.032$ ) and before the second bout ( $P=0.006$ ) compared to that before the first bout. Results of independent t-test indicated that there was a significant difference between the effects of prolonged endurance and resistance

exercises on the number of monocytes 1 hour after the second bout of exercises ( $P=0.02$ ).

## Discussion

The interaction between sport activity and suppression of immune system has not been confirmed for certain. Findings of previous studies indicated an ambivalent response of the immune system to sport activities. This could be attributed to the shear variety of sport events in terms of intensity, length, engagement of other physiological factors such as hormones and also psychological factors (16). Our study did not indicate any significant difference between the effects of two bouts of resistance and endurance exercises on the number of leucocytes, lymphocytes, and neutrophils, but the number of monocytes 1 hour after the second bout was higher in resistance bout compared to endurance bout. These findings are consistent with results of Ronsen et al., (10) who found that the number of leucocytes was higher after 75 minutes of endurance exercise in 70 per cent of

VO<sub>2</sub>max. Li and Gleeson (9) reported the increased number of leucocytes after the second bout of cycling in 60 per cent of VO<sub>2</sub>max. The athletic nature of our participants and equal interval recovery time between exercise bouts could account for the consistency. Similar to our research, Neves et al., (19) reported a change in the number of leucocytes after a bout of resistance exercises. The insignificant change in cortisol concentration accounts for the ineffectiveness of bouts of prolonged resistance exercise on the number of leucocytes.

Our findings are consistent with findings of Ronsen et al., (10, 14) who reported an increase in the number of neutrophils after a two bouts of 75-minute endurance exercise in 70 per cent of VO<sub>2</sub>max. Also, Li and Gleeson (11) reported increase in the number of neutrophils following 2 bouts of 2-hour cycling with 60 per cent of VO<sub>2</sub>max which are consistent with our findings here. They also believed that the significant rise in circulating cortisol accounted for the increased number of neutrophils. Also, Neves et al, (19) reported no change in the number of neutrophils following a bout of resistance exercise.

However, our findings contradicted those in Ronsen et al., (10, 14) who reported an increase in the number of lymphocytes following 2 bouts of 75-minute endurance exercise in 70 per cent of VO<sub>2</sub>max. Also, Li and Gleeson (11) reported increase in the number of leucocytes following 2 bouts of 2-hour cycling exercise in 60 per cent of VO<sub>2</sub>max. The longer endurance exercise could account for the difference between our findings and those in studies cited above. Also, in line with our findings, Neves et al., (19) reported no change in the number of lymphocytes following endurance exercises. Ronsen et al., (10, 14) reported increase in the number of lymphocytes following two bouts of 75-minute endurance exercises in 70 per cent of VO<sub>2</sub>max, a finding that

contradicted our findings here. Li and Gleeson (11), however, found similar results. They reported increase in the number of monocytes following 2 bouts of 2-hour cycling in 60 per cent of VO<sub>2</sub>max. They attributed the increase to higher circulating cortisol following endurance exercise. Neves et al., (19) also found similar results, who reported no change in the number of monocytes following an endurance exercise. It is likely that the change in the number of monocytes is related to the circulating cortisol concentration change.

Our findings however are consistent with findings of Ronsen et al., (10, 14) who reported higher circulating cortisol following two bouts of 75-minute endurance exercise in 70 per cent of VO<sub>2</sub>max. Also, findings of Li and Gleeson (11) were in line with our findings of increased circulating cortisol following 2 bouts of 2-hour cycling exercise in 60 per cent of VO<sub>2</sub>max. Li and Gleeson (11) attributed lower glucose to higher circulating cortisol, however we did not measure glucose in the present research.

Our findings indicated that there was no significant difference between the effects of two bouts of endurance and resistance exercises with the equal recovery time interval on the immunological variables. Thus, it is recommended that athletes and their coaches use equal recovery and resting time between bouts of exercise when they perform endurance and resistance exercises. However, doing exercises with the recovery time of 3 hours in a single day could lead to cumulative effect of individual activities and bring about a catabolic state and weakening of immune system through releasing stress hormones such as cortisol. Given the mixed nature of findings of body of research on the effect of prolonged bouts of exercise on body immune system and on hormone concentration, more controlled and precise studies are highly recommended in the issue.



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